

Energetic constraints and parental care: Is corticosterone indicative of energetic costs of incubation in a precocial bird?

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ABSTRACT

Suppression of the adrenocortical response (e.g., corticosterone release) to an acute stressor is a physiological adjustment thought to decrease the likelihood of avian parents abandoning their nests. However, some periods of parental care, like incubation, are energetically costly, thus corticosterone could increase during these stages to allow incubating parents to utilize energy reserves. Wood ducks (*Aix sponsa*) have ~30 day incubation periods and only the female incubates the eggs. We hypothesized that corticosterone would be important in regulating energy availability during incubation in this species. Because resources invested in reproduction increase with clutch size, we also hypothesized that clutch size would influence plasma corticosterone during incubation. We measured baseline and stress-induced corticosterone in incubating females during early and late stages of incubation. At both stages of incubation all hens had low baseline corticosterone levels. However, we found that stress-induced corticosterone was 105% greater late in incubation than early in incubation. We also detected a significant negative correlation between female body mass and stress-induced corticosterone late in incubation, but not during the early stages of incubation. Furthermore, we found a significant positive relationship between stress-induced corticosterone and clutch size. These lines of evidence support the hypothesis that incubation in wood ducks is energetically costly and corticosterone is important in supporting the energetic demands of incubating hens. Our findings suggest that corticosterone's role in supporting parental care behaviors are dynamic and are influenced by several factors and that there is a greater physiological cost associated with incubating larger clutches.

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Introduction

Birds exhibit tremendous parental care, investing substantial time and resources to rear their young. For many birds the investments that parents make during incubation can have implications for both their current and future reproductive success (Hanssen et al., 2005; Heaney and Monaghan, 1996; Reid et al., 2000a). During incubation birds can have 20–50% higher metabolic rates than non-incubating birds (reviewed in Tinbergen and Williams, 2002), and experimentally increasing clutch size or experimentally altering nest temperatures can affect the size of subsequent clutches, parental survival and immune responses, chick fledging success, and can increase parental metabolic rates during incubation (de Heij et al., 2006; Hanssen et al., 2005; Reid et al., 2000a,b; Tinbergen and Williams, 2002). The energetic cost of incubation can be particularly steep for uniparental incubators that receive no aid from their mate. For instance, female common eiders (*Somateria mollissima*) rarely feed during their

~26 day incubation period and receive no assistance from males. During this time, females lose up to 23–46% of their body mass (Bolduc and Guillemette 2003; Parker and Holm 1990).

Parents navigate the conflicting demands of activities necessary for successful reproduction and those required to maintain their own body condition and survival via a suite of orchestrated physiological signals, including hormones (Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). Hormonal changes that occur during reproduction include alterations in an individual's physiological response to acute stress, most notably the effects of acute stress on corticosterone and prolactin concentrations. Under most circumstances, corticosterone increases and prolactin decreases in response to acute stress in birds (Angelier and Chastel, 2009). Corticosterone is an important hormone in energy regulation and during times of acute stress it shunts resources towards survival, whereas prolactin is a hormone important in promoting parental care behaviors (Angelier and Chastel, 2009; Buntin, 1996; Vleck, 1998). Increases in corticosterone can inhibit parental care behaviors by redirecting resources towards activities necessary for survival and by negatively affecting plasma prolactin concentrations (Angelier et al., 2009; Silverin,

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1986). Thus, according to the parental care hypothesis (Holberton and Wingfield, 2003; O'Reilly and Wingfield, 2001; Wingfield et al., 1995) many birds should maximize their current reproductive success despite potential risks to survival by dampening their physiological response to acute stressors when caring for young in an effort to reduce the likelihood of abandoning their offspring. Such a strategy is particularly relevant if an individual is not likely to have future opportunities to reproduce and thus investing in their current brood has higher value than future reproductive potential (Jessop, 2001; Wingfield and Sapolsky, 2003). The parental care hypothesis supports the idea that seasonal variation in corticosterone results from corticosterone's role in modulating behavior (Romero 2002). Indeed, many birds attenuate hormonal responses to stress during times of parental care, and the degree to which physiological responses to acute stress change can vary among and within species, with factors such as parental age, breeding stage, breeding experience, clutch size, and length of the breeding season influencing the magnitude and persistence of hormonal changes (Angelier et al., 2006; Angelier and Chastel, 2009; Bokony et al., 2009; Goutte et al., 2010; Heidinger et al., 2006; Lendvai et al., 2007; Romero et al., 1997; Schmid et al., 2011; Silverin and Wingfield, 1998). For instance, in breeding pairs of three species of shorebirds the parent exhibiting more parental care had lower stress responses than the parent exhibiting less parental care, whereas both sexes in a fourth species with equivalent biparental care had similar stress responses. (O'Reilly and Wingfield, 2001). Additional data suggest that dampening of corticosterone responses to stress should also vary with the "value" of the brood (the brood value hypothesis), whereby larger broods have higher reproductive value resulting in greater reductions in corticosterone responses to stress (Lendvai et al., 2007).

Although many studies demonstrate that adrenocortical responses to stress decrease during periods of intense parental care and support the parental care hypothesis, caring for young is energetically expensive (Tinbergen and Williams, 2002; Williams, 1996) and glucocorticoids, like corticosterone, are important for maintaining energy balance. Many birds lose considerable weight during the most demanding periods of parental care, and increases in corticosterone could be important in fueling parental behaviors by catabolizing energy stores. Indeed, the energy mobilization hypothesis (Romero, 2002) was one of several hypotheses developed to help explain seasonal variation in baseline and stress-induced corticosterone, whereby animals upregulate corticosterone release during times of high energy demand. For most birds, the highest plasma concentrations of baseline and stress-induced corticosterone correspond with the breeding season (Romero, 2002). Presumably, the relative energy demands of different parental care behaviors vary across the altricial-precocial spectrum, and circulating levels of glucocorticoids should be highest during the most energetically costly parental care stage. For example, in altricial tree swallows (*Tachycineta bicolor*), Bonier et al. (2009) detected a significant negative relationship between clutch size and corticosterone levels in female tree swallows during incubation, but a positive relationship between baseline corticosterone levels of the parent and brood size during chick-rearing, the most demanding parental care stage for this species. In precocial birds, the most demanding period of parental care is incubation, and weight loss can be substantial during this period (e.g., Parker and Holm 1990; Prince et al., 1981) suggesting that elevated glucocorticoids might be important for mobilizing energy stores during incubation. Indeed, a study in magellanic penguins which fast during incubation detected a significant increase in baseline and stress-induced corticosterone in incubating parents from early to late in incubation (Hood et al., 1998; also see Kitaysky, et al., 1999 for a similar finding in kittiwakes). In addition, stress-induced corticosterone late in incubation negatively correlated with body mass in incubating penguins, further suggesting the possible importance of corticosterone in liberating energy stores in precocial birds as they lose mass during incubation.

One additional factor that could influence the energetic demands of incubation is clutch size because incubating larger clutches requires more energy than smaller clutches (Tinbergen and Williams, 2002; Williams, 1996). Furthermore, experimental manipulation of clutch size can result in greater mortality and lower immune responses, brood success, and future reproductive effort of incubating parents (de Heij et al., 2006; Hanssen et al., 2005; Larsen et al., 2003; Reid et al., 2000b), clearly demonstrating that caring for larger broods comes with costs. Contrary to the brood value hypothesis which predicts a negative correlation between stress-induced corticosterone and clutch size (Lendvai et al., 2007), energy constraints can elevate both baseline and stress-induced corticosterone (McEwen and Wingfield, 2003; Romero et al., 2009; Sapolsky et al., 2000) suggesting that parental corticosterone levels could scale positively with clutch size during incubation, particularly in precocial species with uniparental incubation. This effect should be further exaggerated in species that experience brood parasitism because clutch sizes can become particularly enlarged.

In this study we investigated changes in adrenocortical responses to stress from early to late incubation in female wood ducks (*Aix sponsa*, Linnaeus), a waterfowl species with highly precocial young that has an average incubation period of 30 days (range: 27–37 days; Hepp and Bellrose, 1995). This species exhibits uniparental incubation in which only the female incubates the eggs and receives no aid from males during this time (Hepp and Bellrose, 1995). Wood ducks exhibit high incubation constancy (>80%), but they take approximately two breaks a day to forage (Folk and Hepp, 2003; Manlove and Hepp, 2000). Although the energetic deficit female wood ducks experience during incubation is probably not as substantial as it can be for species that undergo fasting during incubation (e.g., Eiders and some penguins), females do tend to lose ~5% of their body mass during the incubation period (Hepp et al., 1990). This decrease in body mass supports the idea that incubation is energetically demanding for wood duck hens and elevated concentrations of corticosterone may be necessary for mediating incubation in this species. To test the energy mobilization hypothesis we measured baseline and stress-induced corticosterone concentrations of incubating female wood ducks at both an early stage of incubation (d3–8) and at a late stage of incubation (d22–28). Specifically, we predicted that 1) corticosterone would increase with incubation period because incubation is energetically costly, and the cumulative energetic deficit should increase with time spent incubating, 2) corticosterone would increase with clutch size because larger clutches require more energy to incubate than smaller clutches, and 3) corticosterone would be highest late in the nesting season because younger females and females of lower body weight nest later in the season than heavier, more experienced females (Hepp and Kennamer, 1993).

Materials and methods

Study species

The wood duck (*Aix sponsa*) is a widely distributed dabbling duck whose breeding range extends throughout much of the eastern half of North America and along the west coast from southern California to British Columbia (Hepp and Bellrose, 1995). Wood ducks are relatively small-bodied (~650–700 g) and occupy a diversity of aquatic habitats, including freshwater marshes, wooded swamps, beaver ponds, and bottomland habitats along major tributaries (Bellrose and Holm, 1994). Female wood ducks nest in tree cavities, but will also use artificial nest boxes, a characteristic that facilitates locating nests and capturing females (Hepp et al., 1987). In our study area in the southeastern U.S., wood ducks initiate nesting in mid-late February and continue nesting until mid-July. Females can produce multiple broods in a breeding season.

Although wood ducks are socially monogamous and begin to form pair bonds in autumn and winter (Ambruster, 1982; Hepp and Bellrose, 1995), only the female cares for the eggs and ducklings (Fredrickson, 1990). Females lay one egg per day and the average clutch

size is 12 eggs (Bellrose and Holm, 1994). However, wood ducks express high levels of brood parasitism with up to 58% of nests being parasitized in some populations. This can frequently result in nests containing > 15 eggs with some nests containing > 30 eggs because more than one female may parasitize a single nest (Hepp and Bellrose, 1995). Often females will deposit eggs in another female's nest before starting their own nest later in the season (Clawson et al., 1979; Heusmann et al., 1980). Night incubation begins when ~75% of the clutch is laid, and 24 h incubation does not begin until egg-laying is complete (Hepp and Bellrose, 1995). During incubation females typically take two recesses per day to forage, once in the morning (avg duration = 86.5 min) and once in the late afternoon (avg duration = 138.7 min; Hepp and Bellrose, 1995). In general older ducks nest 11–19 days earlier than younger hens, but heavier hens, regardless of age, nest earlier than lighter hens (Hepp and Kenamer, 1993).

Study site

Females used in this study were nesting on ephemeral wetlands ($n = 12$) located on the Department of Energy's Savannah River Site (SRS) in west-central South Carolina. Between 4 and 16 nest boxes are located at each wetland, depending on its size ($n = 92$ boxes total). These nest boxes have been available to wood ducks for > 20 years and have been monitored every year by staff and graduate students from the Savannah River Ecology Laboratory and more recently Virginia Tech. All methods were IACUC approved (proposal #11-056-FIW) and animals were collected under the following collection permits: South Carolina Department of Natural Resources G-08-07; US Fish and Wildlife Service MB748024-0.

Monitoring nests and capturing females

We monitored wood duck nests from February 2–May 12, 2011. During this time we checked nest boxes daily to determine when nests were initiated, monitor egg laying, and determine when females began incubating. Once females began incubating we did not disturb the nest until it was time to capture hens for blood collection. Incubating hens were captured by hand and initial blood samples were collected within three min of covering the nest box entry hole. After collecting our first blood sample we collected morphometric data on females (wing chord length, body mass, culmen length, and tarsus length), banded new females, and counted eggs in each nest. We then held females in a cloth bag until 30 min post capture, and then collected a second blood sample. We placed females back on their nest, exited the wetland, and left them undisturbed unless we captured them again late in incubation (see study design below). If we captured a hen a second time, we followed the same sampling protocol. We determined clutch size as the number of eggs present at the time of sampling. Conspecific nest parasitism was common in our system; 22 of 30 nests were parasitized and five of these nests increased in size from early incubation to late incubation due to nest parasitism.

Study design

We collected blood samples from females at two time periods, early in incubation (d3–d8; $n = 23$) and late in incubation (d22–d28; $n = 24$). We considered the start of incubation to be when females began daytime incubation. At both early and late incubation sample dates we collected two blood samples from each hen, a baseline blood sample (<3 min of capture) and a stress-induced blood sample (30 min post-capture) for corticosterone analysis. We were unable to capture some females at both incubation stages because of predation, female abandonment, or because females flushed the nest before they could be captured. The total number of females used in this study was 30.

Blood collection and handling

To obtain blood samples from females we punctured the metatarsal artery using a 26 ½ gauge needle and collected blood (~70–140 μ l at each sample point) in microhematocrit capillary tubes. We immediately placed blood samples on ice until they could be processed (<8 h). Plasma was separated by centrifuging samples at 3.5 g for 5 min. We removed plasma and stored it at -80°C until samples could be analyzed for corticosterone concentrations. All samples were collected between 1000 and 1700 h from March 4–May 12 2011 and assayed for corticosterone in September 2011.

Radioimmunoassay

We determined plasma corticosterone concentrations using extraction and radioimmunoassay (RIA) techniques following the methods of Wingfield et al. (1992). We used the B3-163 antibody (Esoterix Endocrinology, Calabasas, CA) in our assays. To determine sample extraction efficiency we equilibrated each diluted plasma sample (20 μ l plasma in 180 μ l water) overnight with 2000 cpm of tritiated steroid. Each sample was extracted with 4 ml of dichloromethane, dried using nitrogen gas, and resuspended in 550 μ l of phosphate buffered saline. Individual extraction efficiency was determined from 100 μ l of the resuspended sample (mean recoveries were 80.6% for the assay). Two hundred microliters of the resuspended sample was allocated to each of two duplicates for the assay. Then, duplicate samples were compared to a standard curve, which contained known amounts of corticosterone, run with each assay. Intra-assay variation was 6.4% as determined by running standards in the assay. All samples were analyzed in a single assay. Detection limit for the assay was ~1–2 ng/ml. Out of 96 samples, 43 were below detection limit ($n = 42$ baseline samples; $n = 1$ stress-induced sample). For these samples the detection limit of the assay was used as the sample corticosterone concentration for calculating means. In addition, for the single non-detectable stress-induced sample the detection limit of the assay was used in statistical models.

Statistical analyses

All statistical analyses were run in SAS 9.1 (SAS Institute Inc., Cary, NC, USA) or Microsoft Excel and statistical significance was recognized at $\alpha < 0.05$. Where appropriate, we tested for normal distribution of the data and homoscedasticity using Ryan-Joiners and Bartlett's tests, respectively. We used raw data values in statistical analyses, except with analyses that included clutch size. Clutch size was \log_{10} -transformed to better meet assumptions of normality. Because 42 of the 48 baseline samples had corticosterone concentrations below the detection limits of our assay, we limit our statistical comparisons to stress-induced concentrations of corticosterone.

First, we conducted two regression analyses to determine whether it was appropriate to group samples into either early or late incubation. Specifically, we tested for significant relationships between day of incubation and plasma corticosterone concentrations within our early incubation sampling period and our late incubation sampling period. These models confirmed that there was no effect of incubation day within the early or late incubation periods (early [d3–6]: $r^2 = 0.094$, $p = 0.15$; late [d22–28]: $r^2 = 0.017$, $p = 0.54$). Therefore, condensing incubation day into two incubation stages (early and late) was appropriate (Fig. 1). Next, we used a repeated measures ANOVA (SAS Proc Mixed) to test for differences in stress-induced corticosterone between the two stages of incubation.

We further examined how relationships between stress-induced corticosterone and the two stages of incubation could be influenced by other factors using a series of four repeated measures ANCOVAs (SAS Proc Mixed) that included either time of day that samples were collected, date samples were collected, clutch size, or female body mass as the covariate. Each model included incubation stage

as a main effect and the interaction effect between incubation stage and the covariate. In cases where there was no detectable interaction between incubation stage and the covariate ($p \geq 0.13$), we removed the interaction from the model. Finally, to determine whether individual females that were sampled at both incubation stages ($n = 17$) tended to have similar corticosterone profiles during the two stages, we regressed stress-induced corticosterone concentrations late in incubation against their stress-induced corticosterone concentrations early in incubation. We also used linear regression to test for relationships between female body size and clutch size at early incubation and late incubation. In addition, for the individuals that were captured at both the early and late stage of incubation ($n = 17$) we used linear regression to test for relationships between grams of body mass lost and % body mass lost in relation to absolute change in stress-induced corticosterone, % change in plasma corticosterone, and clutch size.

Results

The percentage of baseline samples that had non-detectable corticosterone (i.e., < 1 ng/ml) were similar early in incubation (87%) as late in incubation (79%), indicating that baseline corticosterone concentrations were consistently low in incubating female wood ducks. However, all females had a robust increase in corticosterone following 30 min of handling and restraint (Fig. 2). In addition, female wood ducks had 105% higher stress-induced corticosterone during late incubation as compared to early incubation ($F_{1,17} = 13.87$; $p = 0.001$; Cohen's $f^2 = 0.51$; Fig. 2). Contrary to Lynn et al. (2010), previous capture did not influence female wood duck corticosterone concentrations ($p = 0.81$); the 17 females that were captured twice had similar stress-induced corticosterone concentrations at the second capture (late in incubation; mean \pm SE = 23.2 ± 3.7 ng/ml) as the females that were only captured during the late stage of incubation (25.0 ± 5.5 ng/ml; $n = 6$).

We did not detect a significant effect of time of day on female stress-induced corticosterone ($p = 0.67$). However, we found a significant influence of date on stress-induced corticosterone ($F_{1,16} = 5.77$; $p = 0.03$; Cohen's $f^2 = 0.17$ Fig. 3). Regardless of incubation stage (i.e., early or late), stress-induced corticosterone increased in incubating hens as the nesting season progressed. We also found a significant positive relationship between clutch size and stress-induced corticosterone, regardless of incubation stage ($F_{1,16} = 5.08$; $p = 0.04$; Cohen's $f^2 = 0.14$; Fig. 4). There was also a trend late in incubation, but not early in incubation, of females of low body mass having higher stress-induced

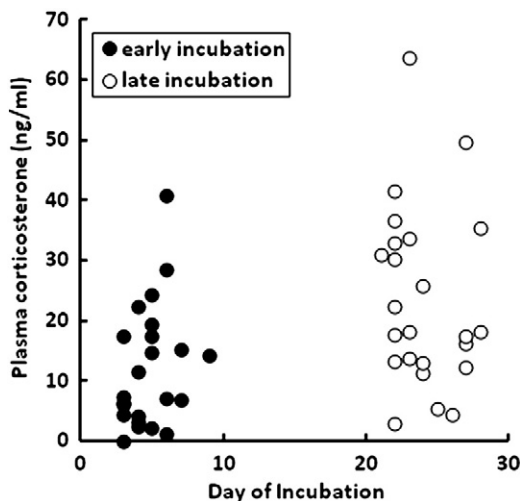


Fig. 1. Stress-induced (30 min post capture) plasma corticosterone of female wood ducks (*Aix sponsa*) plotted against day of incubation.

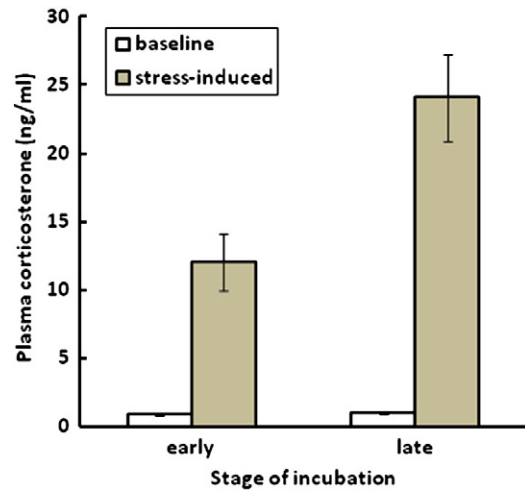


Fig. 2. Baseline and stress-induced (30 min post capture) plasma corticosterone of female wood ducks (*Aix sponsa*) taken early in incubation (d3–6) and late in incubation (d22–28).

corticosterone concentrations than females of high body mass (mass \times incubation stage: $F_{1,15} = 3.35$; Cohen's $f^2 = 0.90$; $p = 0.09$; Fig. 5). For the subset of individuals that were sampled at both incubation stages, there was a significant positive relationship between stress-induced corticosterone concentrations early in incubation and late in incubation ($R^2 = 0.273$, partial eta squared = 0.27; $p = 0.03$; Fig. 6). Female body mass did not correlate with clutch size at either incubation stage (in both cases $R^2 = 0.003$, $p = 0.69$).

Based on the 17 females that were captured at both the early and late stage of incubation average change in body mass (g) and % change in body mass by females during incubation was -16.5 g (range: -70 g to $+20$ g) and -2.77% (range: -11.3% to $+3.5\%$ of initial body mass), respectively. We did not detect a significant relationship between grams of body mass lost or % body mass lost in relation to absolute change in stress-induced corticosterone, % change in stress-induced corticosterone, and clutch size (in all cases $R^2 = 0.12$; $p = 0.17$).

Discussion

Contrary to our predictions, we did not detect changes in baseline corticosterone in our study. Although most baseline samples were below the detection limit, the robust increase in stress-induced titers

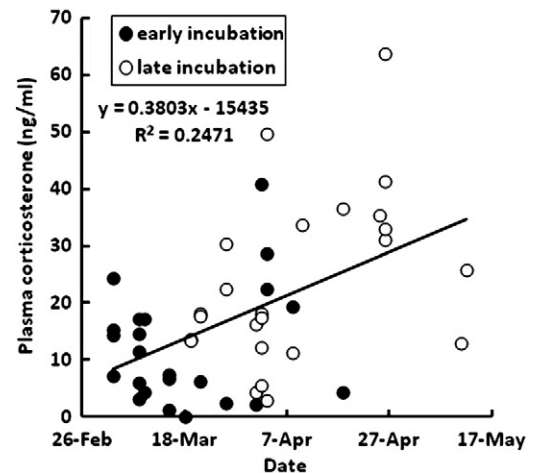


Fig. 3. Stress-induced (30 min post capture) plasma corticosterone of female wood ducks (*Aix sponsa*) taken during early and late stages of incubation plotted against date sample was collected.

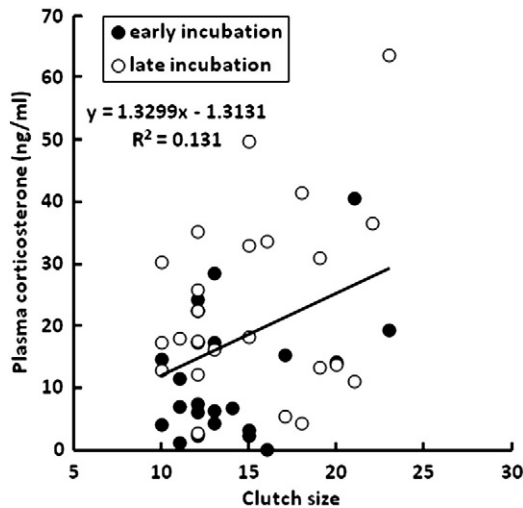


Fig. 4. Stress-induced (30 min post capture) plasma corticosterone of female wood ducks (*Aix sponsa*) taken during early and late stages of incubation plotted against clutch size. The single regression line is fit to all of the data.

suggests that a large increase in baseline titers would have been detectable. Typically, increases in baseline corticosterone are predicted to occur when energy stores need to be mobilized to support daily activities (Landys et al., 2006). Studies of penguins and eiders demonstrate that baseline corticosterone increased 30–190% during incubation (14 to 18 ng/ml, Bourgeon and Raclot 2006; 2.5 to 6 ng/ml, Hood et al., 1998; 2.36 to 6.89 ng/ml, Cockrem et al., 2006), a time of reduced feeding, which was concomitant with 10–19% decreases in body mass. Baseline corticosterone of wood ducks may have experienced similar percent increases in response to factors such as clutch size and duration of incubation, but they never achieved levels that were greater than the limit of detection for our assay (~1–2 ng/ml). It is important to recognize that we were likely sampling female wood ducks during both a diel and seasonal trough in corticosterone levels which could explain why we were unable to detect changes in baseline corticosterone during incubation. We typically sampled females after they had returned from their morning feeding recess when baseline corticosterone levels should be at their lowest. In addition, female wood ducks are also molting during reproduction and baseline and stress-induced corticosterone is often lowest during molt (Romero, 2002). To fully appreciate how female wood ducks

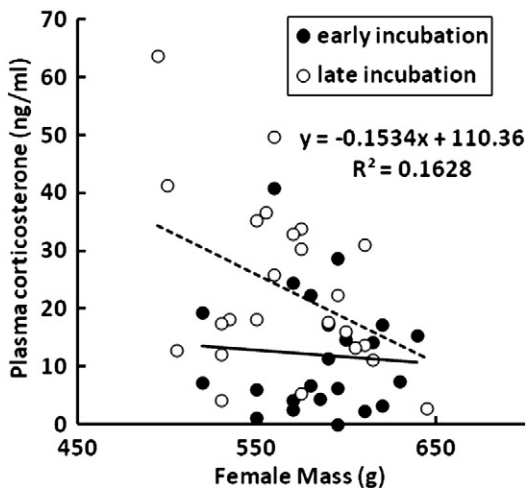


Fig. 5. Stress-induced (30 min post capture) plasma corticosterone of female wood ducks (*Aix sponsa*) taken during early and late stages of incubation plotted against female mass. The solid trend line corresponds with samples taken during early incubation. The dotted trend line corresponds with samples taken late in incubation and with the R^2 value and slope equation.

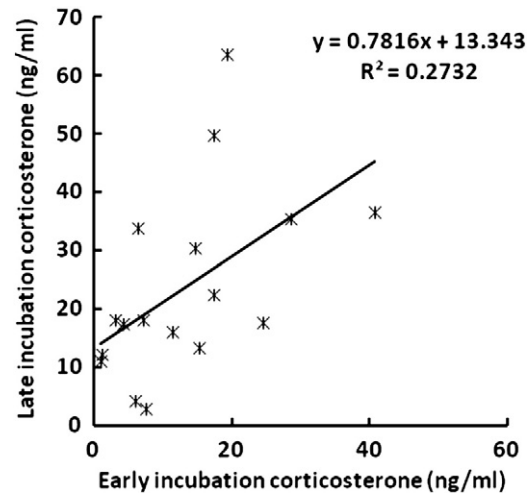


Fig. 6. Stress-induced (30 min post capture) plasma corticosterone concentrations of female wood ducks (*Aix sponsa*) late in incubation plotted against their stress-induced plasma corticosterone concentrations early in incubation.

modulate basal levels of corticosterone both during molt and breeding, data are needed on seasonal variation in baseline corticosterone levels in this species.

Although we did not detect changes in baseline corticosterone during incubation, we did detect changes in stress-induced corticosterone that could be indicative of energetic requirements/demands. Lending support to the energy mobilization hypothesis, we found that stress-induced corticosterone increased with parental investment during incubation, both with time spent incubating and with clutch size (Figs. 1, 2 and 4). Incubation is known to be energetically costly and this cost can increase with clutch size (Erikstad and Tveraa, 1995; Tinbergen and Williams, 2002; Williams, 1996), and stress-induced corticosterone of incubating female wood ducks appears to reflect these energetic costs. Although energy demands are often thought to be modulated by baseline corticosterone, stress-induced corticosterone can also be associated with energetic needs. For instance, stress-induced corticosterone often negatively correlates with body condition and fat scores of birds (Kitaysky et al., 1999; Perfito et al., 2002; Wingfield et al., 1994) suggesting that lower energy stores may require greater stimulation during acute stress for mobilization of energy from stores to occur (McEwen and Wingfield, 2003; Romero et al., 2009; Sapolsky et al., 2000). In addition, some literature suggest that when birds first begin energetically costly behaviors like incubation or migration, energy stores are high and the stress response is dampened to prevent exhausting available stores (Holberton, 1999; Hood et al., 1998; Perfito et al., 2002). Whereas later, mounting a stress response is important for dealing with acute stress, since the animal needs to break down reserves to survive (Hood et al. 1999). Incubation should be the most energetically demanding period of parental care for birds with highly precocial young, and as a result corticosterone may play an important role in mediating these energy demands. In contrast, birds with altricial young or precocial young that require extensive care post-hatching should experience similar increases in corticosterone across the chick-rearing stage which is likely the most energetically costly period of parental care for these species. Indeed, a study in altricial tree swallows found that baseline corticosterone was highest during chick rearing and positively correlated with brood size presumably due to demands of provisioning young (Bonier et al., 2009).

Further support for the energy mobilization hypothesis is the negative relationship between stress-induced corticosterone and female body mass during the latter stage of incubation, while no such relationship existed at the start of incubation (Fig. 5). Our findings are consistent with a study on magellanic penguins, *Spheniscus magellanicus*

(Hood et al., 1998). In penguins, both basal and stress-induced corticosterone were higher later in incubation, and the authors also detected a negative relationship between stress-induced corticosterone and body mass and condition late in incubation, but not early in incubation. Magellanic penguins fast during incubation, thus Hood et al. (1998) proposed that the energetic requirements of incubating penguins were supported by elevated corticosterone levels. Although wood ducks do not fast during incubation they do reduce the amount of time they spend feeding, and nearly all of our females lost weight during incubation (range: -11.3% to $+3.5\%$ of initial body mass; mean: -2.77% change in body mass) suggesting that females may require larger increases in corticosterone during an acute stressor to liberate energy stores during the later stages of incubation. Again similar to our study, although not compared within a single stage of parental care, Perfito et al. (2002) found that baseline and stress-induced corticosterone increased from early to late in the breeding season in harlequin ducks and maximum corticosterone production negatively correlated with female body condition. In addition to liberating energy stores, the higher corticosterone concentrations of birds in poor body condition (Hood et al., 1998; Perfito et al., 2002; this study) could indicate that corticosterone ultimately acts to mediate the trade-off between current reproduction and immediate survival, and subsequently future reproductive opportunities (e.g., body condition hypothesis; Angelier et al., 2009).

One of the most interesting findings in our study was the positive relationship between stress-induced corticosterone and clutch size (Fig. 4). Because brood parasitism commonly results in enlarged clutches in wood ducks, this species is a good model for examining the physiological costs of incubation. In fact, the female incubating the largest clutch of eggs in this study (23 eggs), which all hatched, also had the highest stress-induced corticosterone concentration late in incubation (64 ng/ml). A study in common eider, revealed a similar increase in stress-induced corticosterone but not baseline corticosterone when clutch size was experimentally-increased relative to unmanipulated clutches (Crisuolo et al., 2006). Historically incubation costs were thought to be low in birds (Monaghan and Nager, 1997; Stearns, 1992), thus suggesting that the energy cost of brood parasitism to the parasitized parent in precocial birds would be low relative to altricial birds due to the lower parental care demands of precocial hatchlings. However, our finding that females incubating larger clutches have higher corticosterone concentrations than females incubating smaller clutches suggests that there are physiological costs incurred by females incubating parasitized clutches. In other words, the increased allostatic load associated with incubating a larger clutch will require a more robust stress response when faced with an emergency to allow the animal to cope with the stressor (McEwen and Wingfield, 2003; Romero et al., 2009; Sapolsky et al., 2000). Increased physiological costs associated with larger clutches is consistent with prior studies that showed there are higher energetic demands associated with maintaining optimal incubation temperatures for a larger clutch (Erikstad and Tveraa, 1995; Tinbergen and Williams, 2002; Williams, 1996). In addition, energetic costs could result from increased time spent incubating, because large clutches can take longer to incubate in wood ducks (Hepp et al., 1990). Further studies are needed to better understand the relationships between clutch size, corticosterone, incubation behavior, and energy budgets of incubating birds and implications for future reproduction and survival.

For the most part our data do not support the parental care hypothesis, except in one regard; stress-induced corticosterone was highest later in the season when younger females should be nesting (Fig. 3). The parental care hypothesis predicts that increases in corticosterone in response to acute stress should decrease during periods of parental care, with attenuation being most pronounced during high levels of parental care and in older individuals with low probability of future reproductive success (Angelier et al., 2007a,b, 2009;

Bokony et al., 2009; Goutte et al., 2010; Holberton and Wingfield, 2003; O'Reilly and Wingfield, 2001; Wingfield et al., 1995). In wood ducks, older and heavier females tend to nest earlier in the season (Hepp and Kennamer, 1993) which could explain why stress-induced corticosterone levels of nesting birds are lower earlier in the nesting season. However, we do not know the exact ages of the wood ducks used in our study because we cannot accurately age wood ducks unless they are banded during their first year.

Another contributing factor to the seasonal differences in corticosterone in wood ducks could be increased nest-predation risks. Research in other bird species demonstrated higher levels of corticosterone in birds nesting in areas experiencing high nest predation relative to areas with lower predation pressure (Clinchy et al., 2004; Travers et al., 2010). Similar to other studies on wood ducks (Hepp and Kennamer, 1993), we noted greater nest predation in this study as the nesting season progressed (first predated nest was April 9th). It may be that females in our system are more responsive to stressors later in the season when predation risks are higher. Changes in corticosterone in relation to predation risks would support a preparative role for the modulation of corticosterone during incubation (Romero, 2002; Sapolsky et al., 2000). If energy demands increase with time spent incubating then incubating females may have to spend more time foraging or take more recesses which could leave her susceptible to predators (de Heij et al., 2008), thus making it advantageous to be capable of mounting a strong stress response.

Our findings suggest that corticosterone plays an important role in female wood duck energetics during incubation, with both incubation stage and clutch size affecting the energetic demands females experience when incubating. Many studies have shown that corticosterone decreases during breeding stages that require parental care behaviors and this decrease is thought to decrease nest abandonment. However, our findings suggest that the relationship between corticosterone and parental care may be more complicated than previously believed. The patterns we detected in stress-induced corticosterone lend support to both the parental care and energy mobilization hypotheses as well as a potential preparative role of corticosterone indicating that these hypotheses are not mutually exclusive, and could be incorporated into a more integrative model. For instance corticosterone may play a more dynamic role during reproduction with its modulation being dependent on individual condition, relative importance of current versus future reproduction, environmental conditions, and the relative duration a parent has been engaged in parental care. This perspective predicts that corticosterone is low as individuals transition into parental care stages to permit parental care behaviors, but as relative energy demands (e.g., dependent on clutch size, environmental conditions, body condition) increase so should corticosterone to sustain these energetically costly behaviors. However, to fully reconcile the relationships between parental care behaviors and corticosterone more studies are needed that compare corticosterone levels across multiple stages of breeding and at multiple time points within single stages of breeding. These data will be most revealing when interpreted in relation to prolactin concentrations across and within breeding stages (Riou et al., 2010). For instance, high concentrations of circulating prolactin could help to offset any negative effect that increases in corticosterone may have on parental care (Riou et al., 2010). Lastly, because clutch size positively correlated with stress-induced corticosterone it suggests that energetic demands on parents are greater as clutch size increases. This energetic cost incurred by parents incubating large clutches may act as a constraint on clutch size in many birds (Monaghan and Nager, 1997).

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